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# Sol–gel processed mupirocin silica microspheres loaded collagen scaffold: A synergistic bio-composite for wound healing

Sathiamurthi Perumal [a,b,1], Satiesh kumar Ramadass [a,1], Balaraman Madhan [a,*]

[a] Central Leather Research Institute, Council of Scientific and Industrial Research, Adyar, Chennai 20, India
[b] Academy of Scientific and Innovative Research, Central Leather Research Institute, Council of Scientific and Industrial Research, Adyar, Chennai 20, India

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ABSTRACT

Development of a bio-composite using synergistic combination is a promising strategy to address various pathological manifestations of acute and chronic wounds. In the present work, we have combined three materials viz., mupirocin as an antimicrobial drug, sol–gel processed silica microsphere as drug carrier for sustained delivery of drug and collagen, an established wound healer as scaffold. The mupirocin-loaded silica microspheres (Mu-SM) and Mu-SM loaded collagen scaffold were characterized for surface morphology, entrapment efficiency and distribution homogeneity, *in vitro* drug release, water uptake capacity, cell proliferation and antibacterial activity. *In vivo* wound healing efficacy of the bio-composite was experimented using full thickness excision wound model in Wistar albino rats. The Mu-SM incorporated collagen scaffold showed good *in vitro* characteristics in terms of better water uptake, sustained drug availability and antimicrobial activity. The wound closure analysis revealed that the complete epithelialisation was observed at 14.2 ± 0.44 days for Mu-SM loaded collagen, whereas this was 17.4 ± 0.44 days and 20.6 ± 0.54 days for collagen and control groups, respectively. Consequently, the synergistic strategy of combining mupirocin-loaded silica microspheres and collagen as a Mu-SM loaded collagen dressing material would be an ideal biomaterial for the treatment of surface wounds, burns and foot ulcers.



## 1. Introduction

The upcoming strategy for effective management of surface wounds, burns and foot ulcers is to develop biomaterials based on synergistic approach. Advanced wound healing biomaterials utilizes the knowledge of current drug treatment and tissue engineering substitutes to address various pathological manifestations of acute and chronic wounds. Chronic wounds frequently appear to be "stuck" in the inflammatory phase and can lead to tissue damage if it last too long (Midwood et al., 2004). Although, inflammation is a necessary part of the normal healing process but, presence of macrophage on wound bed can prevent the subsequent proliferative phase (Newton et al., 2004). Bacterial infection is the major underlying reason that prolongs the inflammatory phase. The interplay between the wound infection and inflammatory response continues to be an area of significant and intensive investigation. Surgeons are facing challenge in providing the best wound dressing material with good antimicrobial properly which can minimize the local contamination/infections from the surroundings (Russell et al., 2000). Consequently, therapeutic strategies to target infection and inflammatory phase are critical in the treatment of infected wounds.

Infectious organisms preferentially target the wound beneath the dressing materials leading to a serious infection that requires frequent removal of wound dressing and application of topical antimicrobial therapy (Mi et al., 2002). Therefore, the concept of incorporating an antimicrobial agent into dressing materials was developed and is being investigated for treating acute and chronic wounds. Collagen, a wound healing matrix protein has gained broad clinical acceptance, being seen as a safe material (Ramshaw et al., 2009). Collagen scaffolds impregnated with antimicrobial therapeutics have been reported for successful tissue regeneration and proven to be effective for the treatment of wounds (Schlapp and Friess, 2003; Friess and Schlapp, 2002; Sripriya et al., 2007). However, selection of the antimicrobial drug plays a crucial role as it should combat the pathogens associated with wound chronicity including methicillin resistant *Staphylococcus aureus* (*MRSA*). *MRSA* have become increasingly prevalent as nosocomial pathogens, especially in burn wounds (Rode et al., 1989). Mupirocin is an effective antibacterial agent often used in clinical practice as topical ointment to treat a wide variety of topical wounds including burns and foot ulcers because of its effectiveness against wound pathogens including *MRSA*. Mupirocin had been shown to have reparative effect for operative wounds, burns, skin infections, superinfection of chronic dermatoses, and eradication of nasal

* Corresponding author. Tel.: +91 44 24437217.
*E-mail address:* bmadhan76@yahoo.co.in (B. Madhan).
[1] These authors equally contributed for the work.

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carriage of *S. aureus* (Spann et al., 2004). Moreover, mupirocin is effective in treating both second and third degree burns (Dai et al., 2010).

In clinical situation, appropriate amount of antimicrobial drug should be available at the site of the infection for the requisite duration. On that basis, the effective management of chronic wounds can better be achieved by incorporating antimicrobial drug loaded drug delivery system like microsphere into the collagen scaffold which would provide sustained availability of drug throughout the dressing application period. For preparing such advanced biomaterial, it is essential to choose a drug carrier which is biocompatible for wound application. Silica is one such optimal carrier because of its biocompatibility, biodegradability and exudates adsorption property. Furthermore, silica-based dressing materials have been successfully used for the treatment of burn wounds (Baharestani, 2007). Currently, there is no such biomaterial which utilizes the combination of collagen, mupirocin and silica for the treatment of wounds. Therefore, the goal of the present study is to evaluate the wound healing efficacy of Mu-SM loaded collagen bio-composite and to evaluate its applicability as wound dressing material.

## 2. Experimental

### 2.1. Preparation of Mu-SM and Mu-SM incorporated collagen scaffold

Mupirocin loaded-silica microspheres were prepared by sol–gel method described elsewhere (Radin et al., 2009). In brief, 3 mL of tetraethoxysilane (TEOS), 720 μL of 0.1 M HCl and distilled water were mixed and stirred to form an acid catalyzed sol. The molar ratio, R of total water (including water in 0.1 M HCl) to TEOS was 5. To the acid catalyzed sol, 200 mg of mupirocin dissolved in 200 μL of ethanol was added and cooled down to 4 °C. Subsequently, 0.08 M $NH_4OH$ was added dropwise to adjust the pH to 6.0, which led to the time of gelation between 5 and 10 min. The sol was added dropwise into 100 ml vegetable oil stirred at 300 rpm. The stirring was continued until microspheres precipitated to the bottom of the beaker. The microspheres were filtered, rinsed with water and left to dry overnight at 40 °C. Additional experimental trails were carried out with varying the stirring speed viz, 400 and 500 rpm, to observe its influence on the entrapment efficiency of the drug.

Pepsin solubilized Type 1 collagen was isolated from bovine Achilles tendon according to the procedure described elsewhere (Tanaka et al., 1988). The isolated collagen was extensively dialyzed against 0.05 M acetic acid, freeze dried and stored at 4 °C. For scaffold preparation, 45 mg of lyophilized collagen was dissolved in 15 mL of 0.05 M acetic acid. To the collagen solution, 300 mg of mupirocin loaded microspheres (mupirocin equivalent to 30 mg) and 30 μL triton-X 100 were added followed by homogenization for 15 min using Ultra-Turrax IKA T25 at 12000 rpm to disperse the microspheres homogeneously throughout the solution. The homogenized foam solution was immediately poured into the 90 mm sterile petridish, stored overnight at −70 °C and freeze dried.

### 2.2. Characterization of Mu-SM and Mu-SM loaded collagen scaffold

#### 2.2.1. SEM Morphology and particle size analysis

Scanning electron microscopy (SEM) is an excellent tool to examine the shape and surface morphology of both microspheres and scaffold. Both microspheres and scaffold (5 × 5 mm) were placed onto one side of adhesive stub. The stub was then coated with gold using JEOL-JFC 1600 AUTO COATER and was examined under JEOL-JFC 6360 SEM for qualitative assessment of shape and morphology. The Particle size distribution was analyzed using Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK).

#### 2.2.2. Entrapment efficiency and distribution homogeneity

Encapsulation efficiency of mupirocin loaded in silica microspheres was determined by extracting known amount of microspheres in methanol containing 0.1 M Hydrochloric acid. The sample was stirred for 24 h, filtered and the mupirocin was quantified spectrophotometrically using UV–Visible Cary 100 spectrophotometer. To determine distribution homogeneity, the microspheres loaded scaffold was cut into four equal counters and each counter was subjected to extraction procedure. The spectrophotometric analysis was carried out to determine the distribution homogeneity of mupirocin-loaded silica microspheres.

#### 2.2.3. Physical characterization and FTIR analysis

For water uptake study, the collagen scaffolds with and without Mu-SM were separately immersed in distilled water at room temperature for 1 h. After being removed from the water, they were hung over a table until no water dripped from them and then weighed. The water uptake of the matrices was calculated by the following equation:

$$\text{Water uptake}(\%) = \left[\frac{\text{Ws} - \text{Wd}}{\text{Wd}}\right] \times 100$$

where $W_d$ is the weight of the dry matrix and $W_s$ is the weight of the wet matrix.

Mechanical properties such as tensile strength (MPa) and percentage of elongation at break (%) were measured using a universal testing machine (INSTRON model 1405) at an extension rate of 5 mm/min. Fourier transform infrared (FTIR) analysis was carried out to investigate the chemical interactions between the collagen, silica and mupirocin. The spectra were recorded in the transmittance mode at 2 $cm^{-1}$ resolution, between 4000 and 600 $cm^{-1}$ using Nicolet 360 FTIR Spectrometer.

#### 2.2.4. In vitro mupirocin release

*In vitro* release of mupirocin from microspheres was carried out according to the method described elsewhere (Adhirajan et al., 2009). Mu-SM (300 mg) suspended in 2 ml of release medium was taken in the dialysis tube (MW CO 12,000 D, 16 mm diameter, HiMedia, Mumbai, India) and both ends were tightly tied. Then, the dialysis bag was placed in 200 ml (including 2 ml used for suspending Mu-SM) of physiological synthetic serum electrolyte solution, pH 7.4 maintained at 37 °C, with constant stirring. The release of Mu-SM loaded collagen scaffold was carried out using Franz diffusion model. A 1.5 $cm^2$ scaffold was placed in the upper chamber and 17.5 mL release medium was poured into the lower chamber. These two chambers were separated by a wet dialysis membrane placed over the aperture of Franz diffusion apparatus. Aliquots of 1 mL were withdrawn at various time intervals and replaced by same volume of fresh medium. Mupirocin release was determined spectrophotometrically. Mupirocin of equivalent amount directly loaded onto collagen scaffold served as a control for release comparison.

#### 2.2.5. Fibroblast proliferation assay

The mouse embryonic fibroblasts (3T3-L1) cell line was obtained from National Centre for Cell Sciences (NCCS), Pune, India. The Mu-SM loaded collagen scaffold was seeded with 3T3-L1 fibroblast cells at the density of $2 \times 10^5$ cells/well. The scaffolds were then incubated at 37 °C under 5% $CO_2$ for 3 h to facilitate initial cell attachment. After the initial incubation, 300 μL of medium (Dulbecco's Modified Eagle's Medium (HiMedia, India) supplemented with 10% fetal bovine serum (Gibco, India) were added to the

culture wells and incubated at 37 °C under 5% $CO_2$. Culture media were renewed every 2 days. The cells were harvested at day 1, 3 and 7 post-treatment and cell proliferation was assessed by 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Serum free medium containing MTT (0.5 mg/ml) was added and incubated for 4 h at 37 °C in $CO_2$ incubator. The MTT reaction medium was then discarded and 100 μL of dimethylsulfoxide was added and mixed thoroughly. Spectrophotometric absorbance was measured using ELISA Reader at 570 nm.

#### 2.2.6. Antibacterial activity

The antibacterial activity of Mu-SM incorporated collagen scaffold was examined against four bacterial strains namely *Bacillus subtilis (*MTCC 441*), Methicillin-resistant S. aureus* (*MRSA,* ATCC 25923*), Escherichia coli (*ATCC 25922*)* and *Pseudomonas aeruginosa* (ATCC 27853). All the cultures are sub-cultured periodically and maintained on nutrient agar. The inoculum size of each strain was standardized to $10^4$ bacterial cells/mL for each test organism by adjusting the optical density of the bacterial suspension to a turbidity corresponding to spectrophotometric absorbance of 0.05 at 600 nm. The antimicrobial activity of the Mu-SM (300 mg) was evaluated using the broth dilution assay and sustained antimicrobial activity was determined by counting the colony forming units (CFU). At 24 h and 72 h intervals, 0.1 mL of the sample were withdrawn and diluted two fold with normal saline. From the diluted sample, 0.1 mL was spread on Mueller–Hinton agar plates and incubated for 24 h at 35 ± 2 °C. The sustained antibacterial activity was evaluated by counting the colony forming units.

### 2.3. Full thickness excision wound healing model

Male Wistar rats (body weight range 200–220 g) were used for the study. Animals were acclimatized under standard animal laboratory condition for 7 days before used in the experiment. All experiments were approved by institutional animal ethical committee (Central Leather Research Institute, Chennai) and are in agreement with the guidelines for the proper use of animals for biomedical research. Animals were divided into three groups, each consisting of 15 rats,

- Group 1: Mu-SM loaded collagen scaffold (Test group).
- Group 2: Collagen scaffold (Reference group).
- Group 3: Undressed wound covered with sterile guaze (Control group).

Animals were anesthetised with ketamine (dose 60 mg/kg) by intraperitoneal injection, the dorsal hair was shaved and disinfected. Full thickness wounds measuring $2 \times 2$ $cm^2$ were created by excising the dorsal skin. The materials were applied on excised wounds, covered and tied with absorbent gauze to maintain the position. Animals were sacrificed at day 5, 10 and 15 subsequent to wound creation and wound contraction was measured. The percentage wound reduction was calculated according to the following formula (Park et al., 2003).

$$Cn = [(So - Sn)/So] \times 100$$

where Cn is the percentage of wound size-reduction on days 5, 10 and 15 after treatment, So – initial wound size, Sn – wound size on day 5, 10 and 15 after treatment.

The regeneration tissue were exercised and subjected to histopathological examination. For histopathological investigation, tissue specimen was fixed in 10% neutral-buffered formalin, embedded in paraffin wax, sectioned at 3 μm thickness and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT) stain. Histological interpretation was performed by the pathologist as treatment blinded assessment manner.

## 3. Results and discussion

### 3.1. SEM morphology and particle size analysis

SEM morphology of Mu-SM and Mu-SM loaded collagen biocomposite are shown in Fig. 1. As showed in Fig. 1a and b, the Mu-SM shows spherical shape with smooth surface appearance. The appearance of Mu-SM incorporated collagen scaffold confirmed the homogenous distribution of microspheres throughout the collagen scaffold (Fig. 1d). The presence of interconnected porous mesh like appearance between the collagen fibril would be benevolent for cell attachment, proliferation and migration for tissue regeneration. In addition, porous nature of scaffold would help better oxygen supply to the wounds. Both control collagen (Fig. 1c) and Mu-SM loaded collagen scaffold (Fig. 1d) showed the presence of interconnected pores between the fibril network structures. Particle size distribution analysis revealed that microspheres were in the size range of 6–25 μm and the average mean particle size was 13.5 μm.

### 3.2. Drug loading and distribution homogeneity of Mu-SM in collagen scaffold

The entrapment efficiency of mupirocin loaded in silica microspheres was assessed as a function of speed of stirring during emulsification. The emulsification was carried out at varying speeds viz., 300, 400 and 500 rpm and the entrapment efficiency was observed to be decreasing with higher rpm. At 300 rpm, the entrapment efficiency was found to be 88.26 ± 3.56% whereas; at 500 rpm, the entrapment efficiency is found to be 73.04 ± 3.12%. Hence, Mu-SM prepared at 300 rpm was used for loading onto the collagen scaffold.

The distribution homogeneity was assessed to ensure the content uniformity of the drug throughout the Mu-SM collagen scaffold. The analysis showed that the drug content in four counters were found to be uniform and was in between 98.44% and 101.12%.

### 3.3. Mupirocin release from Mu-SM and Mu-SM loaded collagen scaffold

The release profile of mupirocin from Mu-SM and Mu-SM loaded collagen scaffold are shown in Fig. 2. Franz diffusion model was used for carrying out *in vitro* drug release from the scaffold as it simulates the clinical application of a topical delivery system applied on wound surface. Drug release studies from both the microspheres and microspheres loaded scaffold were carried out to compare the extent of drug release controlled by the scaffold. In addition, directly incorporated mupirocin onto collagen scaffold serves as a control to appreciate the sustained release behavior of silica microspheres. The drug release from the microspheres exhibited biphasic pattern characterized by fast initial release in the first 24 h followed by a slow release phase. In the first 4 h, 24.97 ± 4.43% of drug was released from the microspheres, whereas only 9.53 ± 1.23% of drug was released from scaffold. After 4 h, the drug release from scaffold started increasing and showed 38.57 ± 3.54% release after 12 h compared to 42.67 ± 4.06% release from microspheres. It is clearly evident that the scaffold slowed down the initial drug release which could be due to the initial time consumed for wetting the scaffold under experimental condition. However, more than 50% of drug was released at 24 h from both the microspheres and scaffold. This greater fraction of drug release is essential to control the infection load on wound bed after

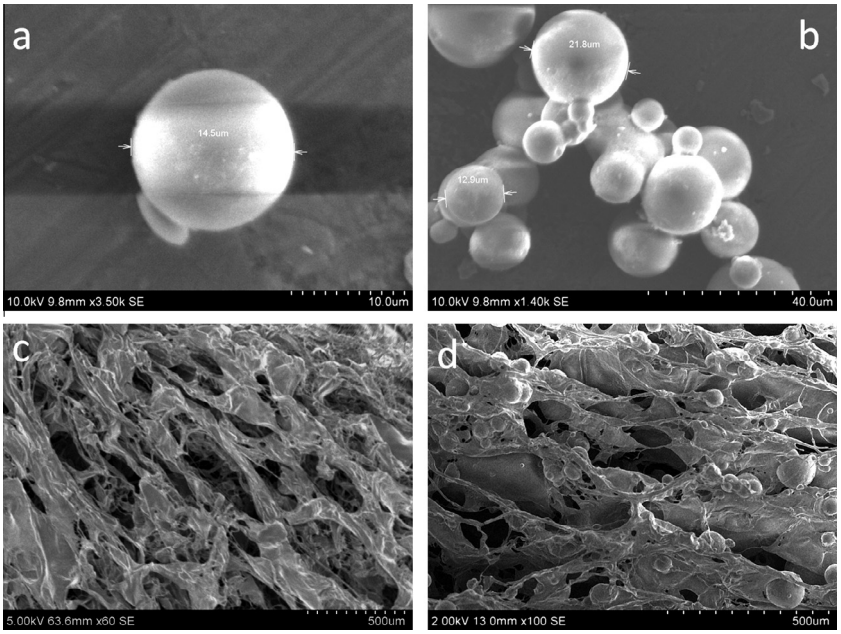


**Fig. 1.** SEM morphology of Mu-SM and Mu-SM loaded collagen scaffold. (a and b) Mu-SM, (c) collagen scaffold and (d) Mu-SM loaded collagen scaffold.

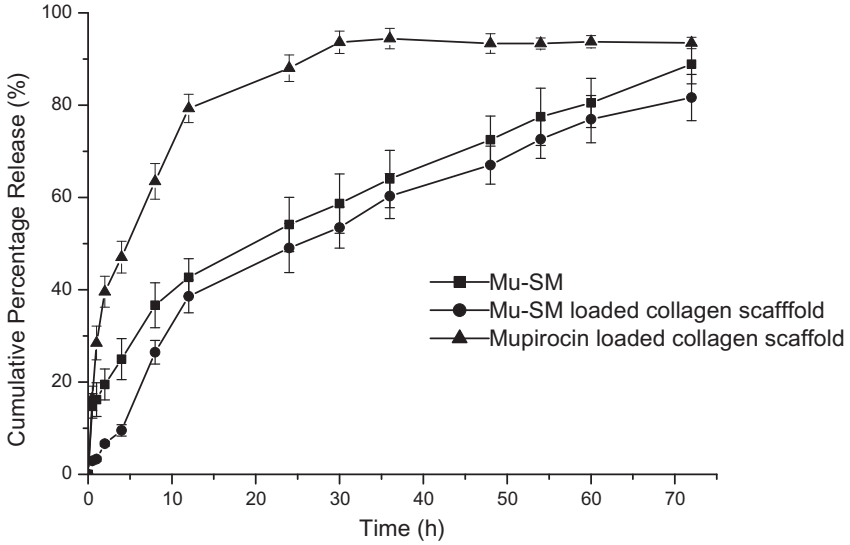


**Fig. 2.** Cumulative release profile of mupirocin from collagen scaffold, Mu-SM and Mu-SM loaded collagen bio-composite.

primary application. Subsequent analysis showed that 72.52 ± 5.12% and 67.01 ± 4.01% of drug was released at 48 h from Mu-SM and Mu-SM loaded scaffold, respectively. The material application holds good if the scaffold is capable of delivering therapeutic agent throughout the period of application. The drug release of 81.65 ± 5.02% at 72 h from the Mu-SM loaded scaffold showed that it is capable of delivering the drug for more than three days for clinical applications. In contrast, directly incorporated mupirocin onto collagen scaffold released 88.01 ± 2.01% of the drug within 24 h. Sustained drug delivery mediated by silica microspheres would be helpful in controlling the infection for a longer period and reducing the frequency of application which leads to faster wound healing and cost effective treatment.

### *3.4. Water uptake, tensile strength and FTIR analysis*

Water uptake capacity of the scaffold is quantified to determine the ability of wound dressing material to adsorb exudate from the wound. It is a crucial property of a wound dressing material especially in case of exudate wounds like severe burns, pressure sores, leg ulcers and decubitus wounds (Gorham, 1991 and Chvapil, 1982). As a result of fluid absorption, rehydration of necrotic tissue of a wound is facilitated, and hence autolytic debridement of the wound is promoted (Zahedi et al., 2010). The Mu-SM loaded collagen scaffold showed significant increase in the weight compared to collagen scaffold (Table 1). It is clearly evident that the water uptake capacity of Mu-SM loaded collagen scaffold showed 12% increase compared to the collagen scaffold. This property could be because of the water adsorbing characteristic of silica microspheres carrier incorporated in collagen. Therefore, silica carrier will not only aid in sustained release of drug but also, act as an exudate adsorbent resulting in better therapy for wound management.

The mechanical property of a wound dressing material is very important, as they are going to be handled by physician while applying onto the wound surface (Muthukumar et al., 2013). Keeping this in mind, tensile strength measurement for Mu-SM loaded

**Table 1**
Water uptake capacity, mechanical characteristics of collagen scaffold and Mu-SM loaded collagen scaffold.

| S. no | Material | Water capacity (%) | Tensile strength (MPa) | Elongation break (%) |
|---|---|---|---|---|
| 1 | Collagen scaffold | 2177.34 ± 32.32 | 3.15 ± 0.12 | 56.50 ± 2.23 |
| 2 | Mu-SM loaded collagen scaffold | 2438.16 ± 37.45 | 2.23 ± 0.11 | 58.58 ± 1.76 |

collagen scaffold was performed and compared with conventionally used collagen scaffold. Collagen scaffolds exhibit slightly higher tensile strength than Mu-SM loaded collagen scaffold (Table 1) and this could be due to the incorporation of silica microspheres between the collagen fibrous structures, thus leading to widening and rupturing of the fibrous network. However, there is no significant difference observed in elongation property between the collagen and Mu-SM loaded collagen composite. These results implicate that the mechanical strength of the developed material is sufficient for handling in clinical settings during application on wound surface.

In FTIR analysis (Fig. 3), mupirocin exhibits absorption at the wave numbers of about 1708 $cm^{-1}$ corresponding to the CO stretching, 1648 $cm^{-1}$ to conjugated CO stretching, 1231 $cm^{-1}$ to ester acetate stretching and 1151 $cm^{-1}$ to C–O–C stretching. Collagen shows characteristic IR bands at 1651 $cm^{-1}$ attributed to amide I CO stretching, 1547 $cm^{-1}$ to amide II NH bending and 1238 $cm^{-1}$ for amide III CN stretching. Empty silica microspheres showed characteristic absorption at 1085 $cm^{-1}$ assigned to Si–O–Si stretching, 940 $cm^{-1}$ to Si–OH stretching, 1635 $cm^{-1}$ to CO bending and 3425 $cm^{-1}$ to OH stretching. Mu-SM loaded collagen showed all major peaks corresponding to mupirocin, collagen and silica, which is indicative of the absence of strong interactions between them.

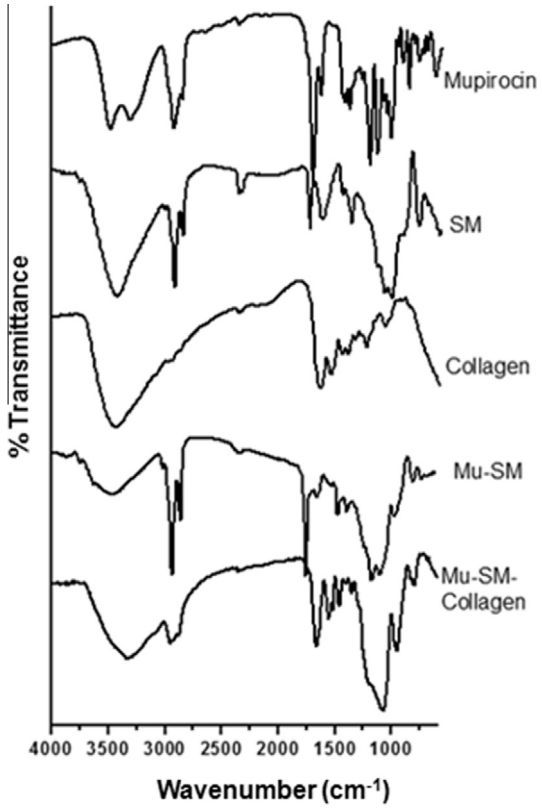


**Fig. 3.** FTIR analysis of collagen, silica microspheres (SM), mupirocin, Mu-SM and Mu-SM loaded collagen bio-composite.

### 3.5. Cell proliferation study

Cell proliferation is a crucial property for wound healing biomaterials to support cell differentiation and tissue regeneration. Collagen based biomaterial remains as a gold standard material for treating wounds because of its cell proliferative and non immunogenic property. MTT based cell proliferation assay revealed that the fibroblast proliferation on collagen and Mu-SM loaded collagen matrix increased significantly compared to the control (Fig. 4 A). On day 1, fibroblast proliferation on collagen matrix was slightly higher compared to Mu-SM loaded collagen matrix. However, this variation is reduced on subsequent sampling (day 3 and day 7) and it is non-significant between the treatment groups. Chang et al. reported that the silica nanoparticles have decreased the dermal fibroblast proliferation at higher concentration but it is non toxic at low doses (Chang et al., 2007). The results clearly indicates that the silica microspheres show minimum influence on cell proliferation but the overall proliferation rate is comparable with that of collagen.

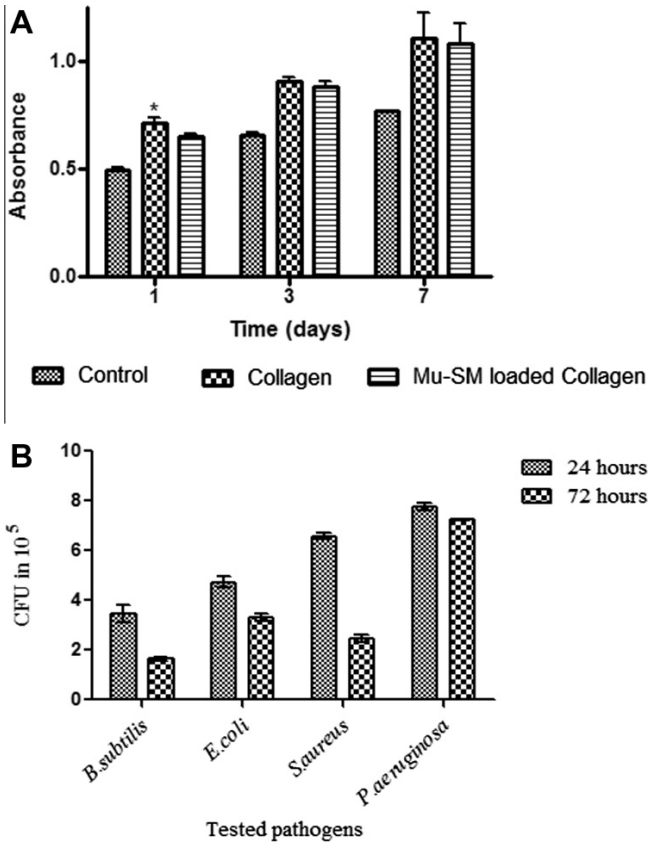


**Fig. 4.** (A) Cell proliferation of 3T3-L1 fibroblast cells seeded on culture plate well (control), collagen scaffold and Mu-SM loaded collagen scaffold. No significant difference between collagen and Mu-SM loaded collagen treatment except day 1 sampling ($p < 0.05$), (B) antibacterial activity of Mu-SM loaded collagen scaffold against wound pathogens. Comparison of bacterial CFU after 24 and 72 h treatment.

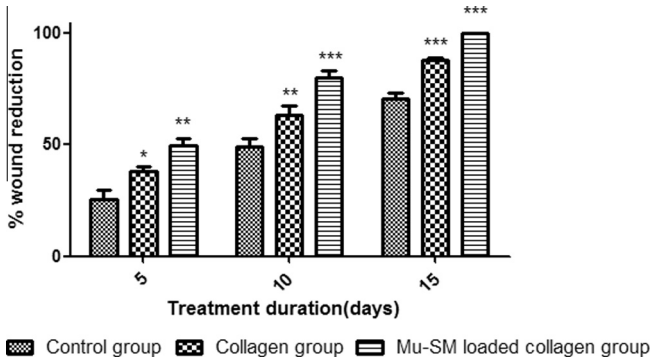


**Fig. 5.** Percentage wound contraction for control (untreated wound), collagen and Mu-SM collagen treatment. Significant difference in the efficacy was observed throughout the treatment duration for both collagen ($p < 0.01$, **) and Mu-SM loaded collagen ($p < 0.001$, ***) compared to the untreated group.

### 3.6. Antibacterial efficacy

Antibacterial activity was examined against four pathogenic strains, which are more prevalent in infectious wound. The Mu-SM-loaded collagen scaffold has been found to be most active against Gram-positive bacteria compared to Gram-negative strain. The developed material exhibited sustained antimicrobial activity over the period of 72 h. The test material showed lesser CFU (colony forming unit) count at 72 h compared to 24 h against all the tested wound pathogens indicating the prolonged antibacterial activity (Fig. 4B). *MRSA* is often found in burn wounds despite of using a silver sulfadiazine and chlorhexidine digluconate as topical therapy (Clarke, 1975 and Inman et al., 1984). *MRSA* may cause serious and even lethal infections associated with the mortality of 20–40% among those clinically infected (Linnemann et al., 1982). The Mu-SM loaded collagen scaffold had shown excellent activity against *MRSA* strain which further emphasizes the therapeutic benefit of Mu-SM loaded collagen scaffold in treating burn wounds especially infected with *MRSA*. Overall, Mu-SM loaded collagen scaffold exhibited significant activity against *MRSA* and *B. subtilis*, moderate activity against *E. coli* and non-significant against *P. aeruginosa*. Therefore, the developed biomaterial could be more effective in treating burns infected with *MRSA*.

### 3.7. Wound closure analysis and histopathology

Collagen scaffold loses its counter shape when applied over the wound bed due to its spongy nature and easy compressibility. The porosity of the material would be affected during such deformation. However, Mu-SM loaded collagen scaffold withstands the compression during application and showed better adhesion on wound surface. Scaffold's porous structure and adherence property helps in cell proliferation, function, and migration (Natarajan et al., 2013). Fig. 5 shows the percentage wound contraction at various time intervals on post-treatment. Mu-SM loaded collagen showed gradual increase in the wound contraction compared to the collagen group throughout the treatment period. However, Mu-SM loaded collagen treatment showed more significant wound reduction during the initial period compared to collagen treatment and control. Additionally, Mu-SM showed highly accelerated granulation tissue formation during the initial post treatment period compared to the reference and untreated group. On day 5 and 10, wound size reduction in Mu-SM loaded collagen treated group increased to 43.56 ± 3.25% and 77.27 ± 2.29% compared to 35.09 ± 2.09% and 64.13 ± 4.33% of collagen treatment, whereas this increased to 25.57 ± 4.03% and 49.37 ± 3.32% in the control group. The complete epithelialisation of Mu-SM loaded collagen treated group was observed to be at 14.2 ± 0.44 days, whereas this is observed to be at 17.4 ± 0.44 days and 20.6 ± 0.54 days for collagen and control group, respectively. The therapeutic combination selection plays a vital role in wound healing and should ideally improve one or more phases of healing. Non-healing chronic wound often stays longer at the inflammatory phase and inflammation plays an important role in fighting infection, clearing debris and inducing the proliferation phase, but prolonged inflammatory phase can be detrimental to the wound healing. Therefore, reducing the duration of inflammatory phase by preventing the bacterial invasion is frequently the major goal in wound care therapy. In addition, an ideal skin substitute requires the properties such as, adherence to wound, good biocompatibility and good mechanical stability (Stadelmann et al., 1998). Mu-SM loaded collagen could be a better candidate in addressing all pathological conditions and reducing the severity of chronic wounds. The better efficacy of this scaffold is due to the synergistic effect of collagen and mupirocin loaded silica, the former facilitating the tissue regeneration

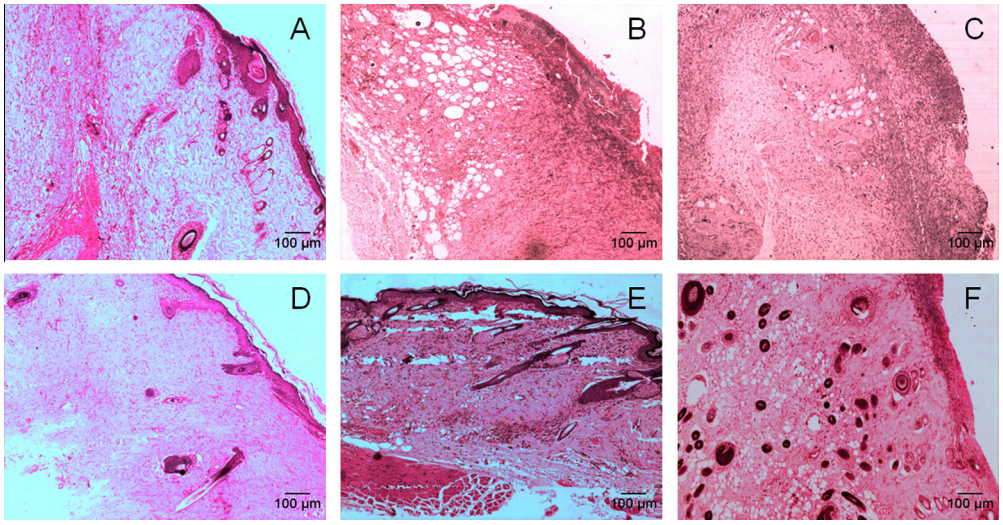


**Fig. 6.** Hematoxylin/eosin-stained section of the regenerated skin tissues (groups 1–3) on day 10. (A) group 1, (B) group 2 and (C) group 3, and day 15, (D) group 1, (E) group 2 and (F) group 3. Group 1 – Mu-SM loaded collagen; group 2 – collagen; group 3 – control. Original magnification is 40× and the scale bar = 100 µm.

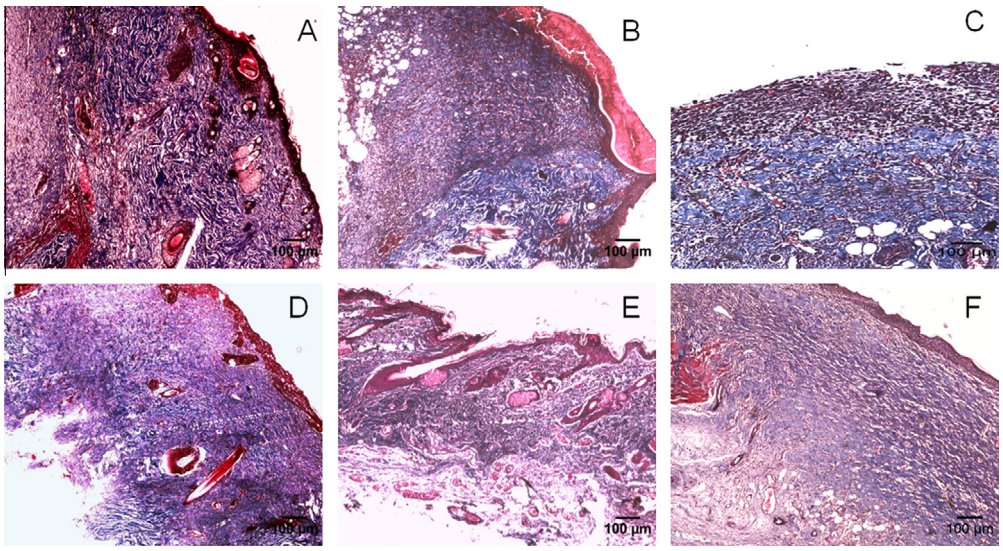


**Fig. 7.** Masson's trichrome stained section of the regenerated skin tissues (groups 1–3) on day 10. (A) group 1, (B) group 2 and (C) group 3, and day 15, (D) group 1, (E) group 2 and (F) group 3. Group 1 – Mu-SM loaded collagen; group 2 – collagen reference; group 3 – control. Original magnification is 40× and the scale bar = 100 µm.

and the latter controlling the inflammation phase and protecting against microbial invasion.

The histological examination on exercised healing tissue was performed using H&E and MT staining to observe the inflammatory response, fibroblast proliferation and collagen deposition. The initial wound goes through a chain of programmed, interdependent responses to the injury, including inflammation, epithelialization, angiogenesis, and accumulation of extracellular matrix (Chen and Abatangelo, 1999). On day 10, Both collagen treatment and untreated group showed moderate inflammatory infiltrate compared to the Mu-SM loaded collagen treatment, which clearly indicates that the antibiotic incorporation in the scaffold would helped in faster healing by preventing the prolonged inflammatory phase. Additionally, epithelialisation at the edge of wound was observed to be good for the Mu-SM loaded collagen group (Fig. 6). The better fibroblast proliferation rate observed with both Mu-SM loaded collagen and collagen treatment and lead to increasing collagen synthesis, which is clearly evident in MT staining (Fig. 7). In addition, Mu-SM loaded collagen treatment exhibited prominent vascularity, which facilitates the oxygen and essential nutrients transfer for tissue regeneration. It is well established that the collagen formation is very important for tissue repair and remodeling (Ruszczak, 2003). This observation was in accordance with the wound contraction measurement at different intervals. On day 15, Mu-SM loaded collagen treatment showed complete epithelialisation with focal acanthosis and adenexal structure, whereas moderate epithelialisation was seen in collagen group and for control (untreated group), defective epithelialisation was observed. Similarly, MT staining revealed increased accumulation of dermal collagen with Mu-SM loaded collagen treatment. The faster epithelial regeneration and increased collagen deposition with Mu-SM loaded collagen treatment could significantly accelerate wound healing compared to collagen treatment. Moreover the faster healing and deposition of collagen did not lead any scar formation in the wounds and hence Mu-SM loaded collagen scaffold would be an effective alternative scaffold for deep wounds.

## 4. Conclusion

In the present study, Mu-SM was prepared using simple sol–gel methodology, loaded onto collagen scaffold with the aim to prevent infection and promote faster healing. The morphological study showed the uniform distribution of microspheres throughout the scaffold. The sustained delivery of mupirocin from silica microspheres would certainly help in combating infection throughout the application period. *In vivo* study revealed that the developed material shown better adhesion property and wound contraction rate. The supporting histological investigation showed better fibroblast proliferation and dermal collagenization. Hence, we conclude that the developed material has better property in terms of delivering the antibacterial drug for a period of about 72 h, excellent water uptake capacity and wound contraction, and thus Mu-SM loaded collagen bio-composite has a potential as an effective wound dressing material applicable for the treatment of infectious and chronic wounds.

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